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Attachable Solvatochromic Fluorophores and Bioconjugation Studies

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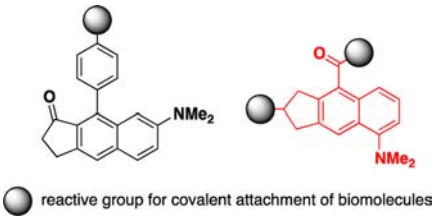
ABSTRACT



The synthesis and utility of attachable cyclopenta[*b*]naphthalene solvatochromic fluorophores related to Prodan are described. Two fluorophores were selected for functionalization and bioconjugation studies. The skeletons were chemically modified to include reactive functional groups and showed minimal alteration of the optical properties when compared to the parent dyes. The functionalized fluorophores were covalently attached to the carboxyl group of a fatty acid, and azido- and thiol-containing amino acids, demonstrating their potential for labeling biomolecules.

Solvatochromic fluorophores represent a fascinating class of organic dyes particularly useful for the study of complex biological systems.[1] Their sensitivity to local microenvironments, low cost, and ease of handling[2] has resulted in the recent applications of these compounds to the field of proteomics and the study of protein folding and protein−protein interactions.[3] Solvatochromic fluorophores are also employed both *in vitro* and *in vivo* as sensitive indicators of lipid organization and dynamics in cellular membranes.[4] Prodan (**1a**) is an example of a commercially available solvatochromic probe widely used for labeling biomolecules and monitoring their structural transformations or interactions (Figure 1).[5] This naphthalene-based dye exhibits desirable photophysical properties such as changes in emission wavelength dependent upon microenvironment polarity, high quantum yield, and

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good photostability. Common derivatives of Prodan include the lipophilic Laurdan (**1b**), the thiol reactive Acrylodan (**1c**), and the amino acid containing Aladan (**1d**, Figure 1).[6] Several solvatochromic fluorophores are commercially available, but development of probes with improved photophysical and chemical properties for selective binding and detection of biological targets remains a field of active research.[7]

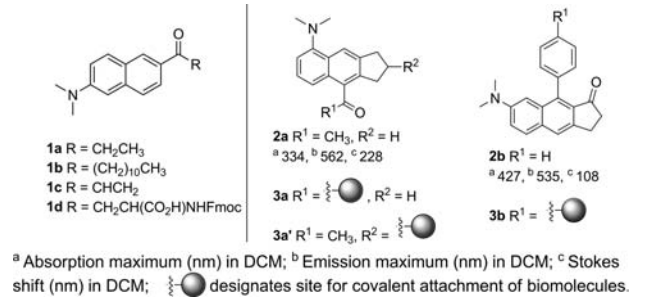

Figure 1. Naphthalene-based solvatochromic fluorophores.

Recently, we reported a concise synthesis of fluorescent dyes **2a** and **2b** (Figure 1). The synthesis employed a dehydrogenative dehydro-Diels–Alder (DDDA) reaction to obtain the keto-naphthalene core and a Buchwald–Hartwig cross-coupling reaction to install the amine group.[8] These fluorophores were shown to absorb and emit light at longer wavelengths and display larger Stokes shifts in ethanol when compared to Prodan while exhibiting similarly high quantum yields and good photostability.[8b] Red-shifted absorption and emission spectra are important because of the reduced phototoxicity in biological systems. In addition, because many Prodan analogs designed for bioconjugation use Prodan as a starting material, we expect that a *de novo* synthesis will afford fluorophores with enhanced biological relevance and versatility.[1f]

To this end, fluorophore **2a** has two functionalization sites that are readily accessible, R^1 and R^2 (Figure 1). Functionalization of R^1 in **3a** with a variety of groups is possible and should afford compounds with the same photophysical properties as parent **2a**. Attachment of functional groups and/or biomolecules to the five membered ring, as in **3a′**, is also predicted to have little effect on the photophysical properties because this ring is not conjugated with the chromophore. Regarding attachment sites for fluorophore **2b**, R^1 on the appended aryl ring is synthetically appealing. In addition, we have previously shown that the aryl ring has little effect on the absorption and emission maxima of the parent dye. We selected a hydroxyl group as a reactive functionality for labeling fluorophores **3a**, **3a′**, and **3b** because of its synthetic versatility, i.e. nucleophilic substitutions, oxidations, and Mitsunobu reactions.[9]

We first set out to examine the functionalization of the cyclopentane group in **3a′**. A microwave-assisted intramolecular DDDA reaction of styrenes **4a**, **4b**, and **4c** afforded cyclopenta[*b*]naphthalenes **5a**, **5b**, and **5c** in 85%, 47%, and 92% yield, respectively (Scheme 1). A low yield for the conversion of **4b** to **5b** was attributed to the bulky *tert*-butyl group. The resulting aryl chlorides **5a**, **5b** and **5c** were subjected to palladium-catalyzed cross-coupling amination conditions to isolate the protected fluorophores **6a**–**d** in 62%, 58%, 71%, and 35% yield, respectively. Reaction conditions for the conversion of **5c** to pyrrolidine **6d** were not optimized. The ketal groups of compounds **6a**–**c** were removed by treatment with 1 N HCl to afford diols **7a**–**c** in 57%, 96%, and 52% yield, respectively. Tetra-*n*-butylammonium fluoride (TBAF, 2 equiv) in THF was used to deprotect the TBS groups of substrate **6d** to afford **7c** in quantitative yield.

Absorption and emission maxima of **6a**–**d** and **7a**–**c** were measured in dichloromethane (DCM) revealing interesting trends. Changes to the amine and ketone groups influence the photophysical properties of these fluorophores, as evidenced by the emission maxima for **6a** (566 nm), **6b** (527 nm), and **6d** (581 nm). However, variations to the diol moiety had almost no effect on the optical properties of these dyes. In fact, the ketal derivative **6a**, the TBS protected compound **6c**, and the free diol **7a** showed almost identical fluorescence emission maxima (566, 564, and 567 nm respectively) and only slight changes in the absorption maxima were observed.

The diol group of **7a**–**c** was considered for the fluorescent labeling of carboxyl groups. To demonstrate this, the fatty acid derivative **8** was obtained through a coupling reaction of **7b** with 10-undecenoic acid and dicyclohexyl carbodiimide (DCC, Scheme 1). Despite the slightly inferior photophysical properties of **7b** when comparing it to **7a** and **7c**, the *tert*-butyl group increases the lipophilicity of this series of compounds and may serve to enhance its potential as a membrane probe. The optical properties of fluorophore **8** were found to be comparable to that of substrate **7b** with an absorption maximum of 324 nm, an emission maximum of 531 nm, and a Stokes shift of 207 nm in DCM. This unusual fatty acid derivative **8** is being examined for its potential to study membrane structure.

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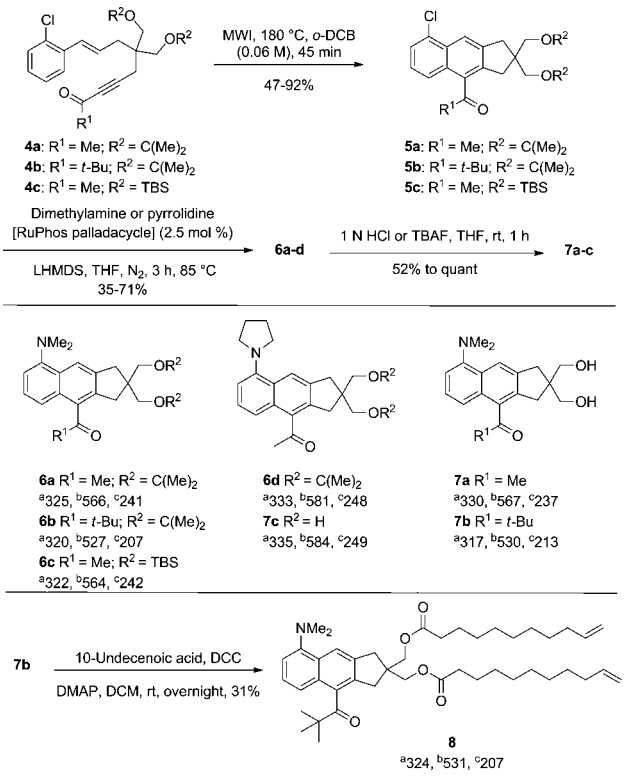
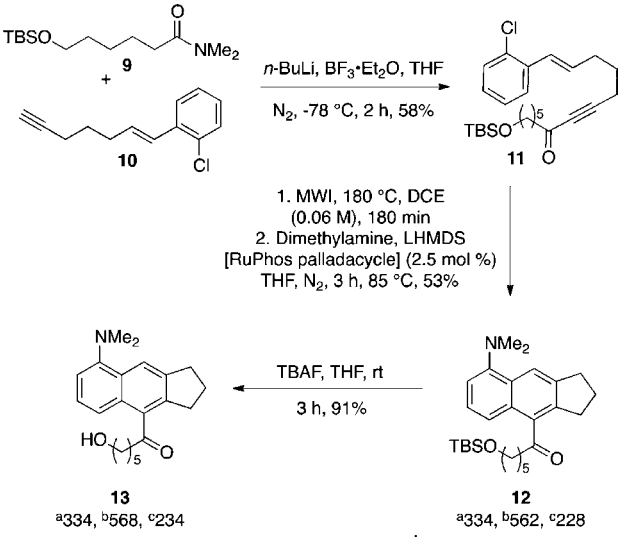
Scheme 1. Synthesis of Fluorescent Diols **7a**–**c**, Their Photophysical Properties, and Fatty Acid Labeling



[a] Absorption maximum (nm) in DCM. [b] Emission maximum (nm) in DCM. [c] Stokes shift (nm) in DCM

Scheme 2. Synthesis of Attachable Dye **13**



[a] Absorption maximum (nm) in DCM. [b] Emission maximum (nm) in DCM; [c] Stokes shift (nm) in DCM.

To widen the applicability and to show the versatility of these dyes as labels for biological targets, our efforts turned to the labeling of **3a** (Figure 1). This was accomplished by reacting amide **9** and the lithium acetylide of alkyne **10**[10] to produce the DDDA precursor **11** in 58% yield (Scheme 2). Subjecting **11** to the DDDA reaction conditions followed by a Buchwald–Hartwig cross-coupling reaction generated the TBS-protected fluorescent compound **12** in a 53% overall yield. Deprotection of the TBS group with TBAF afforded the attachable fluorophore **13** in 91% yield. This convenient synthetic protocol allows for the preparation of additional fluorophores with tethers of varying lengths and conformationally mobility between the carbonyl and the reactive hydroxyl group. With regards to optical properties, the TBS-protected and hydroxyl derivatives **12** and **13** showed absorption and emission maxima in DCM comparable to those observed for fluorophore **2a**.

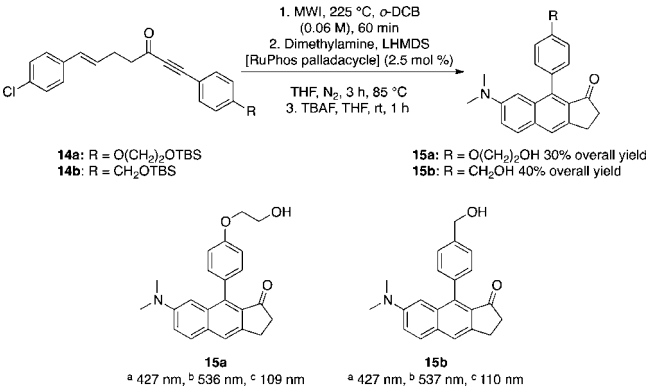
The final labeling strategy is depicted as **3b** (Figure 1). Analogs of fluorophore **2b** are especially attractive because this fluorophore displays an absorption maximum in the visible region of the electromagnetic spectrum (425 nm).[8b] In a manner entirely analogous to the preparation of other DDDA precursors, **14a** and **14b** were prepared, isolated, and subjected to microwave irradiation to produce fluorescent dyes **15a** and **15b** in 30% and 40% yield for the three steps (Scheme 3). These compounds displayed similar optical properties when compared with dye **2b**.

The versatility of the reactive fluorophore **15b** was demonstrated by its conversion into the maleimide derivative **17** employing a two-step protocol involving a Mitsunobu reaction to form the protected maleimide **16**. A thermal retro-Diels–Alder reaction of **16** releases the thiol reactive maleimide group of **17** in 75% yield (Scheme 4). The fluorescent adduct **17** reacted in 10 min with *N*-Boc-L-cysteine ethyl ester to afford conjugate **18** as a (1:1) mixture of diastereomers. Oxidation of **15b** with Dess-Martin periodinane (DMP) gave aldehyde **19** in 67% yield, which was converted into alkyne **20** by treatment with the Bestmann–Ohira reagent. Substrate **20** was successfully employed in a copper-catalyzed click-reaction with *N*-*tert*-butoxycarbonyl-L-β-azidoalanine methyl ester to yield the covalently linked amino acid **21** (Scheme 4). The maleimide derivatives **16** and **17**, the cysteine adduct **18**, the alkyne **20**, and triazole **21** all displayed similar photophysical properties to the parent dye **15b**. Aldehyde **19** is the only compound that exhibited a significantly red-shifted fluorescence emission maximum when compared to **15b** (580 nm vs 537 nm). Finally, **15b** was converted into the fluorescent fatty acid analog **22** (Scheme 5). Derivative **22** maintained all the photophysical characteristics of its precursor **15b** with an absorption maximum in the visible region, a fluorescence emission maximum of 538 nm, and a Stokes shift of 113 nm in DCM.

In conclusion, attachable cyclopenta[*b*]naphthalene solvatochromic fluorophores structurally related to Prodan were synthesized through the functionalization of fluorescent compounds previously reported by our group.

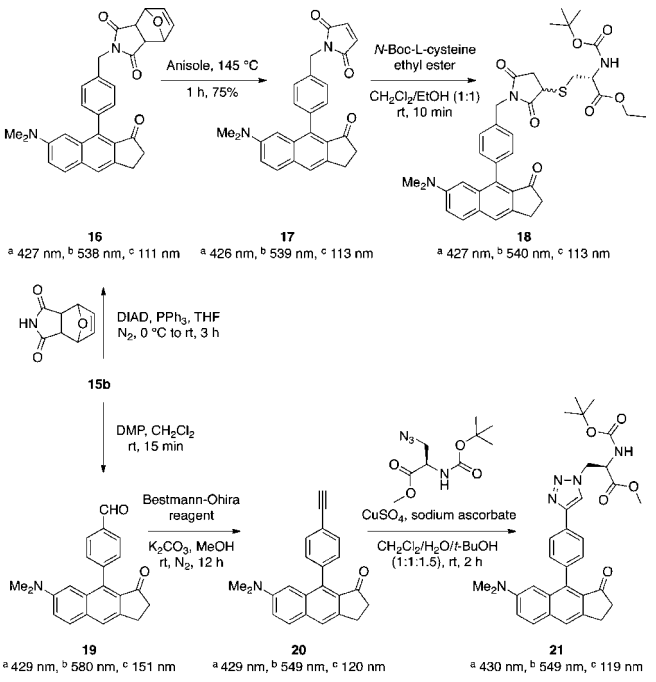
(10) For more details on the synthesis of substrates **9** and **10**, see Supporting Information.

Scheme 3. Synthesis of Dyes **15a** and **15b**



[a] Absorption maximum (nm) in DCM. [b] Emission maximum (nm) in DCM. [c] Stokes shift (nm) in DCM.

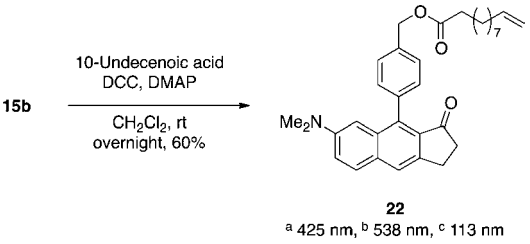
Scheme 4. Synthesis of the Unnatural Fluorescent Amino Acids **18** and **21**



[a] Absorption maximum (nm) in DCM. [b] Emission maximum (nm) in DCM. [c] Stokes shift (nm) in DCM.

Three different sites for structural modification were considered to avoid altering the optical properties of the fluorophores. Utilizing the cyclopentane moiety of these dyes, fluorescent diols were obtained. Probes incorporating conformationally mobile or rigid monohydroxyl-functionalized linkers were also prepared. All fluorophores maintained the photophysical properties of their parent compounds showing enhanced solvatochromism when compared to Prodan. Finally, fluorescent lipid analogs and unnatural amino acid derivatives were prepared starting from the newly synthesized dyes, demonstrating their potential as versatile labels for biomolecules. We expect that these fluorophores will be of general utility in the study of lipid dynamics in cellular membranes and the detection of protein-binding interactions.[11] Future studies are directed toward expanding this chemistry-driven approach to prepare fluorophores with enhanced chemical properties such as multifunctionality and/or increased solubility in buffered aqueous solutions.

Scheme 5. Synthesis of the Fluorescent Fatty Acid Analog **22**



[a] Absorption maximum (nm) in DCM. [b] Emission maximum (nm) in DCM. [c] Stokes shift (nm) in DCM.

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Supporting Information Available. Detailed experimental procedures and characterization data for all new compounds; fluorescent emission spectra for compounds **6a**–**d**, **7a**–**c**, **8**, **12**, **13**, **15a**–**b**, **16**–**22**; solvatochromic spectra for compounds **7b**, **13**, **15b**, **21**, and **22**; quantum yields and extinction coefficients for compounds **7b** and **15b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.